

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
15 January 2004 (15.01.2004)

PCT

(10) International Publication Number
WO 2004/004639 A2

- (51) International Patent Classification⁷: **A61K** MG, MK, MN, MX, NO, NZ, OM, PH, PL, RO, SC, SG, TN, TT, UA, US, UZ, VN, YU, ZA.
- (21) International Application Number: PCT/US2003/020751 (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- (22) International Filing Date: 2 July 2003 (02.07.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 60/393,189 2 July 2002 (02.07.2002) US
- (71) Applicant (for all designated States except US): SMITHKLINE BEECHAM CORPORATION [US/US]; One Franklin Plaza, Philadelphia, PA 19103 (US).
- (72) Inventor; and
- (75) Inventor/Applicant (for US only): NESTA, Douglas, P. [US/US]; 709 Swedeland Road, King of Prussia, PA 19406 (US).
- (74) Agents: LOCKENOUR, Andrea, V. et al.; Smithkline Beecham Corporation, Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).
- (81) Designated States (national): AE, AG, AL, AU, BA, BB, BR, BZ, CA, CN, CO, CR, CU, DM, DZ, EC, GE, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MA, MG, MK, MN, MX, NO, NZ, OM, PH, PL, RO, SC, SG, TN, TT, UA, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)
- Declarations under Rule 4.17:
- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for all designations
 - as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AU, BA, BB, BR, BZ, CA, CN, CO, CR, CU, DM, DZ, EC, GE, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MA, MG, MK, MN, MX, NO, NZ, OM, PH, PL, RO, SC, SG, TN, TT, UA, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)
 - of inventorship (Rule 4.17(iv)) for US only
- Published:
- without international search report and to be republished upon receipt of that report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: A NOVEL STABLE FORMULATION

(57) Abstract: The present invention relates to a stable formulation for huC242-DM1, an antibody conjugated to cytotoxic agent.

WO 2004/004639 A2

A Nov 1 Stable Formulation

FIELD OF THE INVENTION

5 The present invention relates to a stable formulation for *huC242-DM1*, an antibody conjugated to cytotoxic agent.

BACKGROUND OF THE INVENTION

10 In the past ten years, advances in biotechnology have made it possible to produce a variety of proteins for pharmaceutical applications using recombinant DNA techniques. Because proteins are larger and more complex than traditional organic and inorganic drugs (i.e. possessing multiple functional groups in addition to complex three-dimensional structures), the formulation of such proteins poses special problems.

15 For a protein to remain biologically active, a formulation must preserve intact the conformational integrity of at least a core sequence of the protein's amino acids while at the same time protecting the protein's multiple functional groups from degradation. Degradation pathways for proteins can involve chemical instability (i.e. any process which involves modification of the protein by bond formation or cleavage resulting in a

20 new chemical entity) or physical instability (i.e. changes in the higher order structure of the protein). Chemical instability can result from deamidation, racemization, hydrolysis, oxidation, beta elimination or disulfide exchange. Physical instability can result from denaturation, aggregation, precipitation or adsorption, for example. The three most common protein degradation pathways are protein aggregation,

25 deamidation and oxidation. Cleland et al *Critical Reviews in Therapeutic Drug Carrier Systems* 10(4): 307-377 (1993).

 Included in the proteins used for pharmaceutical applications are antibodies. An example of an antibody useful for therapy is a murine antibody C242. *See*. EP 528,527B1. *huC242-DM1* is a tumor-activated immunotoxin under development by

30 GlaxoSmithKline plc as a treatment for antigen-expressing tumor types (lead indication pancreatic or PMP cancer). It consists of a humanized antibody of C242, *huC242*, conjugated to DM1, a new derivative of maytansinoid. There have been many reports on both C242-DM1 and *huC242-DM1*. *See* for example, *Proc. Natl. Acad. Sci. USA*, Vol. 93, pp 8618-8623, 1996; *Current Opinion in Molecular Therapeutics*

35 3(2):198-203, 2001.

SUMMARY OF THE INVENTION

Accordingly, the invention provides a stable aqueous pharmaceutical
5 formulation of *hu*C242-DM1 (the immunoconjugate) comprising the immunoconjugate
concentration range ~1-20 mg/mL) in a buffer maintaining the pH in the range of ~5.8-
6.2 (50 mM succinic acid, pH 6.0), and containing sucrose (~5% w/v); this formulation
is suitable for subsequent lyophilization to create a stable dosage form.

Further provided is a stable frozen formulation for monoclonal antibody
10 C242, comprised of the monoclonal antibody protein (concentration range ~1-30
mg/mL) in a buffer maintaining the pH in the range of ~5.8-6.5 (50 mM succinic acid,
pH 6.0), and containing sucrose (~5% w/v).

Further contemplated in the above formulations is the presence of a
stabilizing surfactant, in order to confer additional stability to the starting solutions of
15 each product such that they may not then require storage under frozen or freeze-dried
conditions.

These and further aspects of the invention will be apparent to those skilled in
the art.

20 DETAILED DESCRIPTION

A "stable" formulation is one in which the antibody or immunoconjugate (both
herein referred also simply as protein), as the case may be, therein essentially retains
its physical stability and/or chemical stability and/or biological activity upon storage.
25 Various analytical techniques for measuring protein stability are available in the art
and are reviewed in Peptide and Protein Drug Delivery, 247-301, Vincent Lee Ed.,
Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. Adv. Drug Delivery
Rev. 10: 29-90 (1993), for example. Stability can be measured at a selected
temperature and other storage conditions for a selected time period.

30 A protein "retains its physical stability" in a pharmaceutical formulation if it
shows no signs of aggregation, precipitation and/or denaturation upon visual
examination of color and/or clarity, or as measured by UV light scattering or by size
exclusion chromatography.

A protein "retains its chemical stability" in a pharmaceutical formulation, if
35 the chemical stability at a given time is such that the protein is considered to still

retain its biological activity as defined below. Chemical stability can be assessed by detecting and quantifying chemically altered forms of the protein. Chemical alteration may involve size modification (e.g. clipping) which can be evaluated using size exclusion chromatography, SDS-PAGE and/or matrix-assisted laser desorption ionization/time-of-flight mass spectrometry (MALDI/TOF MS), for example. Other types of chemical alteration include charge alteration (e.g. occurring as a result of deamidation) which can be evaluated by ion-exchange chromatography, for example.

An antibody "retains its biological activity" in a pharmaceutical formulation, if the biological activity of the antibody at a given time is within about 20% (within the errors of the assay) of the biological activity exhibited at the time the pharmaceutical formulation was prepared as determined in an antigen binding assay, for example.

"Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable regions correspond to those of a non-human immunoglobulin and all or substantially all of the

FR regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., Nature 321:522-525 (1986); Riechmann et al, Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992); US patent no. 5,639,641.

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (e.g. principally residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain

variable domain; Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) and/or those residues from a "hypervariable loop"(e.g. principally residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia Lesk J. Mol. Biol. 196:901-917 (1987)). "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

The humanized C242 has variable heavy and light chain amino acid sequences (SEQ ID NO: 1 and 2, respectively) as shown below.

10

SEQ ID NO:1

QVQLVQSGAEVKKPGETVKISCKASDYTFYYGMNWKQAPGQGLKWMGWIDTTTGE
 PTYAKFKQGRIAFSLETSASTAYLQIKSLKSEDTATYFCARRGPYNWYFDVWGQGTTVT
 15 VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
 QSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE
 LLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
 REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
 TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYS
 20 KLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK.
 SEQ ID NO:2

25

DIVMTQSPLSVPVTPGEPVSISSKSLLSNGNTYLYWFLQRPQGQSPQLLIYRMSNLV
 SGVPDRFSGSGSGTAFTLRLISRVEAEDVGVYYCLQHLEYPFTFGPGTKLELKRVAAPS
 FIFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
 LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC.

Technologies in making *hu*C242-DM1 are described in US Patent Nos 5,208,020; 5,552,293; 5,639,641; and EP528,527.

30

The antibody which is to be formulated is preferably essentially pure and desirably essentially homogeneous (i.e. free from contaminating proteins etc). "Essentially pure" antibody means a composition comprising at least about 90% by weight of the antibody, based on total weight of the composition, preferably at least about 95% by weight. "Essentially homogeneous" antibody means a composition comprising at least about 99% by weight of antibody, based on total weight of the composition.

35

The symbol "~" means "about".

*hu*C242-DM1 to be formulated has not been subjected to prior lyophilization and the formulation of interest herein is an aqueous formulation. An aqueous formulation for *hu*C242-DM1 is prepared comprising ~1-30 mg/mL of *hu*C242-DM1 in a pH-buffered solution. The buffer of this invention has a pH in the range from about

40

5.8 to about 6.2, preferably about pH 6.0. Examples of buffers that will control the pH within this range include acetate (e.g. sodium acetate), succinate (such as sodium succinate), gluconate, histidine, citrate and other organic acid buffers. The buffer concentration can be from about 1 mM to about 100 mM, preferably from about 50 mM. The preferred buffer is succinic acid (about 50 mM), pH 6.0.

A polyol, which acts as a tonicifier and may stabilize *hu*C242-DM1, is included in the formulation. In preferred embodiments, the polyol is a nonreducing sugar, such as sucrose or trehalose. Preferred polyol is sucrose in about 5% w/v.

10 A surfactant can also be added to the *hu*C242-DM1 formulation. Exemplary surfactants include nonionic surfactants such as polysorbates (e.g. polysorbates 20, 80 etc) or poloxamers (e.g. poloxamer 188). The amount of surfactant added is such that it reduces aggregation of the formulated immunoconjugate and/or minimizes the formation of particulates in the formulation and/or reduces adsorption. For example, 15 the surfactant may be present in the formulation in an amount from about 0.001% to about 0.5%, preferably from about 0.005% to about 0.2% and most preferably from about 0.01% to about 0.1%. The addition of Pluronic F68, can also be conceived in case where a solution dosage form was desired.

The stabilizing formulation for antibody C242 is prepared comprising ~1-30 mg/mL of C242 in a pH-buffered solution. The buffer of this invention has a pH in the range from about 5.8 to about 6.5, preferably about pH 6.0. Examples of buffers that will control the pH within this range include acetate (e.g. sodium acetate), succinate (such as sodium succinate), gluconate, histidine, citrate and other organic acid buffers. The buffer concentration can be from about 1 mM to about 100 mM, preferably about 20 50 mM, depending, for example, on the buffer. The preferred buffer is succinic acid (about 50 mM), pH 6.0. An polyol, which acts as a tonicifier and may stabilize C242, is included in the formulation. In preferred embodiments, the polyol is a nonreducing sugar, such as sucrose or trehalose. Preferred polyol is sucrose in about 5% w/v. Preferably the formulation will stabilize C242 for 2 years or longer under 25 -70°C frozen storage during the interim between initial antibody manufacture and conjugation to form *hu*C242-DM1.

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the

invention. All literature and patent citations are incorporated herein by reference.

SPECIFIC EMBODIMENTS

5 A variety of challenging stability problems were encountered during the development of a novel therapeutic monoclonal antibody (mAb) C242 (immunoconjugate) and its immunoconjugate *hu*C242-DM1. These challenges were related primarily to degradation in the form of aggregation (soluble and insoluble) of the protein while in solution, and were resolved via formulation studies and dosage
10 form design. Pre-formulation studies were designed to identify the appropriate pH environment for the stability of the mAb with a minimum of additional formulation excipients. Inclusion of surfactants was examined in order to assess any effects on stability. Sucrose served as a bulking agent, as well as, a cryoprotectant for lyophilization cycle development. Prospective solution formulations were tested in
15 order to determine sensitivities to freeze/thaw cycling, vigorous shaking, stress storage, and light. The protein formulations were subjected to a battery of analyses to assure the potency, purity, and quality of the material, which included, among others pH, appearance, UV/VIS, SDS-PAGE, SEC, ELISA, Bioassay, and cIEF. A final formulation of 50-mM succinic acid, pH 6.0, containing 5.0% sucrose was shown to
20 confer a sufficiently stable environment for a lyophilized immunoconjugate dosage form. However, it was determined that, the addition of a surfactant, such as Pluronic F68, should be considered in the case where a solution dosage form was desired.

What is claimed is:

1. A stable aqueous formulation of *hu*C242-DM1 suitable for subsequent lyophilization comprising *hu*C242-DM1 in the concentration range of about 1 to 20 mg/mL, in a buffer maintained at pH in the range of about 5.8 to 6.2, and sucrose in about 5% w/v.
2. The formulation of claim 1 in which pH is maintained at 6 with between 1 to 100mM succinic acid.
3. The formulation of claim 2 in which the concentration of succinic acid is at 50mM.
4. A stable frozen formulation for monoclonal antibody C242, comprised of C242 in the concentration range of about 1 to 30 mg/mL in a buffer maintained at pH in the range of about 5.8 to 6.5, and sucrose in about 5% w/v.
5. The formulation of claim 4 in which pH is maintained at 6 with between 1 to 100mM succinic acid.
6. The formulation of claim 5 in which the concentration of succinic acid is at 50mM.

SEQUENCE LISTING

<110> SmithKline Beecham Corporation

<120> A Novel Stable Formulation

<130> P51355

<140> unknown

<141> 2003-07-02

<150> 60/393,189

<151> 2002-07-02

<160> 2

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 449

<212> PRT

<213> human

<400> 1

Gln	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Glu
1				5					10					15	
Thr	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Asp	Tyr	Thr	Phe	Thr	Tyr	Tyr
			20					25					30		
Gly	Met	Asn	Trp	Val	Lys	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Lys	Trp	Met
		35					40					45			
Gly	Trp	Ile	Asp	Thr	Thr	Thr	Gly	Glu	Pro	Thr	Tyr	Ala	Gln	Lys	Phe
	50					55					60				
Gln	Gly	Arg	Ile	Ala	Phe	Ser	Leu	Glu	Thr	Ser	Ala	Ser	Thr	Ala	Tyr
65				70					75					80	
Leu	Gln	Ile	Lys	Ser	Leu	Lys	Ser	Glu	Asp	Thr	Ala	Thr	Tyr	Phe	Cys
				85					90					95	
Ala	Arg	Arg	Gly	Pro	Tyr	Asn	Trp	Tyr	Phe	Asp	Val	Trp	Gly	Gln	Gly
			100					105					110		
Thr	Thr	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe

115	120	125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu		
130	135	140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp		
145	150	155
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu		
165	170	175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser		
180	185	190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro		
195	200	205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys		
210	215	220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro		
225	230	235
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser		
245	250	255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp		
260	265	270
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn		
275	280	285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val		
290	295	300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu		
305	310	315
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys		
325	330	335
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr		
340	345	350
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr		
355	360	365
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu		
370	375	380
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu		
385	390	395
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys		
405	410	415
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu		
420	425	430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 435 440 445
 Lys

<210> 2

<211> 219

<212> PRT

<213> human

<400> 2

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Val Pro Val Thr Pro Gly
 1 5 10 15
 Glu Pro Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
 20 25 30
 Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
 35 40 45
 Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Val Ser Gly Val Pro
 50 55 60
 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
 65 70 75 80
 Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Leu Gln His
 85 90 95
 Leu Glu Tyr Pro Phe Thr Phe Gly Pro Gly Thr Lys Leu Glu Leu Lys
 100 105 110
 Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
 115 120 125
 Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
 130 135 140
 Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
 145 150 155 160
 Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
 165 170 175
 Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
 180 185 190
 Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
 195 200 205
 Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215